United States Department of Agriculture
Food Safety and Inspection Service
Office of Public Health and Science
Laboratory QA/QC Division
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Microbiology Laboratory Guidebook
Notice of Change

Chapter **new**, revised, or archived: MLG 4C.00

Title: FSIS Procedure for the Use of the BAX System PCR Assay for Screening *Salmonella* in Ready-to-Eat Meat and Poultry Products and Pasteurized Egg Products

Effective Date: 2/17/03

Description and purpose of change(s):

The use of a rapid screening procedure potentially reduces report-out time for true negative samples by 24 hours. FSIS has validated use of this commercial PCR based screening procedure for processed meat and poultry products and pasteurized egg products. All samples identified as potentially positive for *Salmonella* by these tests are subject to cultural confirmation as described in MLG 4 "Isolation and Identification of *Salmonella* from Meat, Poultry and Egg Products".

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Revision: Original	Replaces: NA	Effective: 2/17/03

Procedure Outline

4A.1 Introduction

4A.1.1 General

This method describes the use of a commercial PCR-based screening procedure as described in MLG 4, Section 4.4.5 to screen test Ready-to-Eat meat and poultry products and pasteurized egg products for *Salmonella.* All samples identified as potentially positive for the presence of *Salmonella* by this test are subject to cultural confirmation as described in MLG 4.

4A.1.2 Limits of Detection

For this method, *Salmonella* detection limits are determined to be better than 1 cfu/g in a 25g sample.

4A.2 Safety Precautions

CDC guidelines for the handling of BioSafety Level 2 organisms should be followed whenever live cultures of *Salmonella* are used. All available Material Safety Data Sheets (MSDS) must be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms used in the

analysis. The personnel who will handle the material should read all MSDS sheets, and all MSDS requirements should be followed.

4A.3 Quality Control Procedures

4A.3.1 Culture Controls

See MLG 4, Section 4.3.1 for a description of the culture controls.

4A.3.2 Sterility Control

Additionally, always prepare at least one "blank" (incubated but un-inoculated pre-enrichment/ enrichment broth) to provide a sterility control for the process.

4A.4 Equipment, Reagents, and Media

In addition to equipment, reagents and media used in analysis of samples as described in MLG 4, the following materials will be needed.

a. PCR tube holder (Qualicon)
b. Cell lysis tube cooling block (Qualicon) held at 5 ± 3°C
c. Techne DB-2A, or equivalent, heating block set at 37 ± 2°C
d. Techne DB-2A, or equivalent, heating block set at 95 ± 2°C
e. Repeating pipettor to deliver 200 ± 20 µl, and sterile tips
f. Pipettor to deliver 5 ± l µl, and sterile disposable filtered tips
g. Pipettor to deliver 150 ± 15 µl, and sterile disposable filtered tips
h. Eight-channel pipettor to deliver 50 ± 5 µl, and sterile disposable tips
i. 12 X 75 mm Falcon 352063, or equivalent, tubes
j. Cell lysis tubes and caps, cell lysis tube rack and box (Genemate 8 strip tubes, ISC Bioexpress, T-3120-5)
k. Pipettor and 5 ml pipettes
l. BAX® System PCR Assay for Screening *Salmonella* kit (Qualicon # 17710608) held at 5 ± 3°C

4A.5 Sample Preparation and Primary Enrichment

Perform sample preparation and pre-enrichment as described in MLG 4, Sections 4.5.1 through 4.5.4, 4.5.8 and 4.5.9 with the exception of incubation time in BPW. The incubation time in BPW for BAX® System PCR Assay for Screening *Salmonella* analysis of Ready-to-Eat meat and poultry products and pasteurized egg products is 18-24h.

4A.6 The BAX® System for Screening *Salmonella* Test Procedure

Follow the current BAX® User's Guide for preparing reagents, performing the test, and reading the results. The equipment must be set up, operated, and all records documented according to laboratory work instructions.

4A.7 Interpretation of Results

a. Samples that test BAX® -negative will be reported as negative. Cultural analysis will continue as per MLG 4, Section 4.5.3.d-i, of a sample BPW pre-enrichment that tests BAX® -positive, BAX® -indeterminate, or has a BAX® signal-error result.

b. In analytical runs where both positive controls test negative, the reserve samples will be retested beginning with sample preparation and enrichment. In analytical runs where one of the positive controls tests negative, the laboratory shall grow the control cultures and continue cultural analysis of all samples by proceeding with isolation and purification steps as per MLG 4, Section 4.5.3.d-i.

4A.8 Completion of Testing if BAX® Unavailable

If circumstances (e.g. a power outage or equipment failure) do not allow testing using the BAX® system, the laboratory shall, if possible, continue cultural analysis of all samples by proceeding with isolation and purification steps as per MLG 4, Section 4.5.3.d-i.

4A.9 Selected References

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 1999. BioSafety in Microbiological and Biomedical Laboratories, 4th ed. U.S. Government Printing Office, Washington, D.C. also found on the internet at: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

BAX® System PCR Automated Detection for Bacterial Screening User Guide, Dupont Qualicon.